A Temperature Responsive Biopolymer for Mercury Remediation

JAN KOSTAL, ASHOK MULCHANDANI, KATIE E. GROPP, AND WILFRED CHEN*

Department of Chemical and Environmental Engineering, University of California, Riverside, California 92521

Tunable biopolymers based on elastin-like polypeptides (ELP) were engineered for the selective removal of mercury. ELP undergoes a reversible thermal precipitation within a wide range of temperatures and was exploited to enable easy recovery of the sequestered mercury. A bacterial metalloregulatory protein, MerR, which binds mercury with an unusually high affinity and selectivity, was fused to the ELP to provide the highly selective nature of the biopolymers. Selective binding of mercury was demonstrated at an expected ratio of 0.5 mercury/biopolymer, and minimal binding of competing heavy metals (cadmium, nickel, and zinc), even at 100-fold excess, was observed. The sequestered mercury was extracted easily, enabling continuous reuse of the biopolymers. In repeating cycles, mercury concentration was reduced to ppb levels, satisfying even drinking water limits. Utility of the biopolymers with mercury-contaminated Lake Elsinore water was demonstrated with no decrease in efficiency. The nanoscale biopolymers reported here using metalloregulatory proteins represent a "green" technology for environmentally benign mercury removal. As nature offers a wide selection of specific metalloregulatory proteins, this technology offers promising solutions to remediation of other important pollutants such as arsenic or chromium.

Introduction

Mercury is highly toxic to the nervous system, particularly the developing nervous system of a fetus or young child (*1*). Because of the lack of knowledge about mercury's toxicity and potential environmental impact, factory effluents in the past were commonly routed into the surrounding areas. As a result, many sites are contaminated with high levels of mercury that is dangerous for wildlife and human populations. There is a need to develop efficient, economical, and ecological alternatives to current methods of treatment of aqueous streams, which include chemical precipitation (*2*) and sorption to activated carbon or ion-exchange resins (*3*).

The search for an "ideal" sorbent that can be used for mercury remediation has been hindered by the desired combination of high affinity, selectivity, and ease of use (*4*). Nanoscale materials have been gaining increasing interest in the area of environmental remediation because of their unique physical, chemical, and biological properties. Synthetic polymeric materials with increased affinity, capacity, and selectivity have been devised; however, the cost of ultrafiltration and the toxic nature of the synthetic polymers render them undesirable for large-scale mercury remediation (*5*). Preferably, recovery of polymer−metal complexes can be achieved by simple changes in process conditions, and environmentally benign materials can be employed.

Genetic and protein engineering have emerged as the latest tools in polymer chemistry for the construction of nanoscale materials that can be controlled precisely at the molecular level. Unlike the statistical nature of step and chain polymerization reactions, biopolymers are specifically pre-programmed within a synthetic gene template that can be precisely controlled over chain length, composition, sequence, and most importantly properties. Elastin-like polypeptides (ELP) are biopolymers consisting of repeating pentapeptide VPGVG (*6*) that can undergo a reversible phase transition from the water-soluble forms into aggregates upon increasing the temperature. The transition temperature (T_t) can be controlled by the chain length and peptide sequence (*7*) and is also responsive to pH, ionic strength, pressure, and covalent modifications of amino acid residues (*8*). ELP has been successfully fused to other peptides or proteins while retaining the temperature responsive property as well as the functionality of the fusion partner (*9*, 10). ELP can be overexpressed in *E. coli* to high yields (*11*), and the protein can be easily purified to homogeneity by taking advantage of the temperature responsive character (*9*).

Many bacteria develop resistance to heavy metals by inducing the expression of an array of resistance proteins. The affinity of these metalloregulatory proteins is typically in the 10^{-8} M range, but the clear advantage is their specificity. For example, the binding affinity of MerR (*12*), a 15.8 kDa regulatory protein used for controlling the expression of enzymes responsible for mercury detoxification, is several orders of magnitude higher for mercury than other heavy metals (*13*). These metalloregulatory proteins are therefore ideal for providing the specificity and affinity of the biopolymer sorbents. In this work, we present a general method for selective removal of mercury by generating tunable biopolymers composed of ELP and MerR. The ELP-fusion technology provides an easy and efficient way to recover and recycle the sequestered mercury. The framework presented should pave the way to designing technologies for the selective removal of other important pollutants such as arsenic and chromium based on their respective metalloregulatory proteins (*14*).

Experimental Section

General Methods. DNA manipulations were performed according to standard methods (*15*). All cloning steps were carried out in *E. coli* JM109. The high fidelity *Pfu* polymerase (Promega, Madison, WI) was used for PCR. DNA sequencing was carried out to verify nucleotide sequences of the recombinant genes.

Plasmid Construction. The DNA encoding MerR protein was obtained by PCR using plasmid pT7KB as the template (*16*), which contained part of the *mer* operon from the transposon Tn501 (*17*). The 5′ and 3′ PCR primers were GGGTGGCATGGAAAACAATTTGG and CGCGGATCCCTAAG-GCATAGCCGA. The 451 bp PCR fragment was digested with *Bam*HI and inserted into the plasmid pET-Ela68-h6 (*10*), which was digested with *Sma*I and *Bam*HI. The resulting plasmid pET-Ela67-merR contained all sequences necessary for expression of the ELP-MerR biopolymer using the T7 polymerase system. Next, pET-Ela67-merR was digested with *Sma*I, and a double-stranded linker GGGCGGTGGTAGCG-GCGCCGGTGGCGCAGGCTCTGG was inserted between the two protein domains creating plasmid pET-Ela67-L-merR.

To construct an expression vector for ELP153MR, plasmids pJAN08 and pJAN08-Ela153, constructed to facilitate cloning of multimeric VPGVG-encoding sequences (Kostal et al.,

* Corresponding author phone: (909)787-2473; fax: (909)787-5696; e-mail: Wilfred@engr.ucr.edu.

manuscript in preparation), were used. pJAN08 was digested with *Age*I, blunt-ended with *Pfu* polymerase, and digested with *Bam*HI. A 3982 bp fragment was ligated with a 481 bp fragment obtained by digesting pET-Ela67-L-merR with *Sma*I and *Bam*HI. The resulting plasmid pJAN08-L-merR was digested with *Nde*I and *Xma*I and ligated with a *Nde*I-*Xma*I bp fragment of plasmid pJAN08-Ela153. A *Nde*I-*Pst*I fragment coding for the ELP153MR biopolymer was inserted into the expression vector pET38b+ (Novagen, Madison, WI), generating the expression vector pET-Ela153-L-merR.

Production and Purification of Biopolymers. For protein expression, plasmids (pET-Ela67-L-merR, pET-Ela153-L-MerR, and pET-Ela78) were introduced into *E. coli* BLR(DE3) (Novagen, Madison, WI). All cultivations were carried out in terrific broth (TB) media (*15*) supplemented with either 100 μg/mL ampicillin (pET-Ela67-L-merR and pET-Ela78) or 30 μg/mL kanamycin (pET-Ela153-L-MerR) at 400 rpm for 48 h at 30 °C. The cells were then harvested by centrifugation, washed in 0.9% NaCl, and resuspended in phosphate buffer (pH 7) containing 50 mM 2-mercaptoethanol. The cells were lysed with a French press, and cell debris was removed by centrifugation for 30 min at 30 000g. The biopolymer was purified from the cell extract by three cycles of inverse temperature transition (*18*). For each cycle, the sample was heated to 30 °C and centrifuged at 30 000g at 30 °C, and the pellet containing the biopolymer was dissolved in ice-cold 50 mM potassium phosphate buffer pH 7 containing 50 mM 2-mercaptoethanol. Purity of the purified protein was verified by SDS−PAGE electrophoresis (*19*) followed by silver staining (Bio-Rad, Hercules, CA), and the molecular weight was confirmed by MALDI-TOF mass spectrometry (DE-STR System, PE Biosystems, Foster City, CA). Concentration of the biopolymers was determined spectrophotometrically at 215 nm. Purified biopolymers were stored at −80 °C in 50 mM potassium phosphate buffer pH 7 containing 50 mM 2-mercaptoethanol.

Characterization of Phase Transition Behaviors. The transition temperature of the biopolymers was measured in a 96-well microplate reader. Two hundred microliters of sample were added in each well, and the optical density was followed at 655 nm in a microplate reader BIO-RAD 3550-UV equipped with temperature control, from 20 and 40 °C. The temperature of transition (T_t) was determined as the temperature at which the optical density reached half of its maximum.

Characterization of Mercury Binding. Initial metal binding experiments were performed in the following buffers: 10 mM Tris-Cl pH 8.0; 50 mM potassium phosphate pH 7.0; and 50 mM sodium acetate pH 6.0 and 4.0. Metal binding experiments in the presence of other metals or chelators, and the recycling experiment, were carried out only in the 50 mM sodium acetate buffer pH 4.0. All buffers were degassed before the experiments. Typically, 10 nmol of biopolymer was heat precipitated from the storage buffer and dissolved in 500 μL of the appropriate buffer on ice. After addition of the required amount of metals ($HgCl_2$, $CdCl_2$, $ZnCl_2$, and $NiCl_2$, all from Fisher Scientific, Fair Lawn, NJ) and incubation for 1 h, the samples were heat precipitated as described above. The pellet, dissolved in concentrated nitric acid at room temperature, and the supernatant were used for metal analyses. Mercury was analyzed by cold-vapor atomic absorption spectroscopy (Coleman model 50B Mercury Analyzer System). The detection limit of this method was approximately 0.1 nmol of mercury in up to 95 mL of solution (1 nM). All other metals were analyzed by flame atomic absorption spectrophotometry (Shimadzu AA6701). For binding experiments in the presence of either chelators or complexing agents, 5 mM EDTA or 2-mercapoethanol was added with the metals. A similar saturation binding experiment was performed with artificially contaminated Lake

TABLE 1. Properties of Biopolymers Used in This Study

symbol	protein sequence	amino acids	kDa
ELP67MR[a]	MEF(VPGVG)$_{67}$-VP-lin1-MerR	498	44.7
ELP153MR[b]	MGP(GVGVP)$_{153}$-lin2-MerR	933	80.1
ELP78	MEF(VPGVG)$_{78}$	393	32.4

[a] lin1 = GGGSGAGGAGSGGG. [b] lin2 = GVGPGTG GGGSGGTG.

Elsinore water (a gift from Dr. M. Andersen from UCR). For the recycling experiments, 50 nmol of ELP153MR were mixed with 20 mL of binding buffer (50 mM sodium acetate pH 4.0) containing 4.36 nmol of mercury. After precipitation, the supernatant was removed for mercury analysis. The resulting pellet was mixed with 2.5 mL of the extraction buffer (binding buffer + 50 mM 2-mercaptoethanol). The regenerated biopolymers were recovered by heating and centrifugation and redissolved in ice-cold buffer. Subsequent cycles were repeated using the same procedure.

For the mercury removal experiments with real water sample, 4.36 nmol of was added to 20 mL of Lake Elsinore water. After thorough mixing, 50 nmol of ELP153MR was added and incubated for 1 h. After precipitation, the pellet and supernatant were subjected to mercury analysis.

Results

Design and Synthesis of the ELP-MerR Biopolymers. In a previous study, ELP-based biopolymers using a polyhistidine tag as the metal chelating domain were generated, demonstrating the possibility of easy purification and regeneration for many repeating cycles (*10*). However, the use of histidine clusters offers no selectivity, low affinity, and a narrow working pH range. To address these problems, we exploited the high specificity and affinity of metalloregulatory proteins toward their cognate heavy metal species as a novel chelating-domain for an improved biopolymer design. Because the transition properties of ELP are a strong function of the chain length, initial designs were focused on obtaining biopolymers that would remain soluble under the desired temperature and pH conditions for mercury removal but aggregate in response to a small environmental stimulus (either salt addition or an elevated temperature). To satisfy these requirements, ELPs consisted of 67 and 153 VPGVG repeats were selected based on their estimated T_t of 39 °C and 33 °C, respectively (*10*). A mercury-responsive metalloregulatory protein, MerR, was fused to the C-terminal to generate biopolymers, ELP67MR and ELP153MR (Table 1). A similar ELP78 biopolymer without any chelating domain was used for comparison.

The biopolymers were produced using a modified induction procedure as described previously (*10*). Up to 800 mg/L of purified biopolymers was obtained by cultivation in a rich nutrient broth medium for 48 h without the addition of IPTG. Thanks to the temperature responsive properties of the ELP domain, the biopolymers were easily purified by inverse temperature cycling to homogeneity as judged by the presence of a single band on SDS−PAGE (Figure 1). The correct molecular weights of the purified biopolymers were independently verified by MALDI-TOF mass spectrometry (data not shown).

Phase Transition Behaviors of the Biopolymers. Since the ELP concentration is known to affect the inverse phase transition behaviors (*9*), the T_t of different biopolymers was characterized as a function of concentration to determine the conditions that will provide a desired transition around 25 °C for easy aggregation and recovery of biopolymer−mercury complexes. Turbidity measurements were used to determine the onset of folding and aggregation, and the value of T_t was defined as the temperature at which 50% turbidity
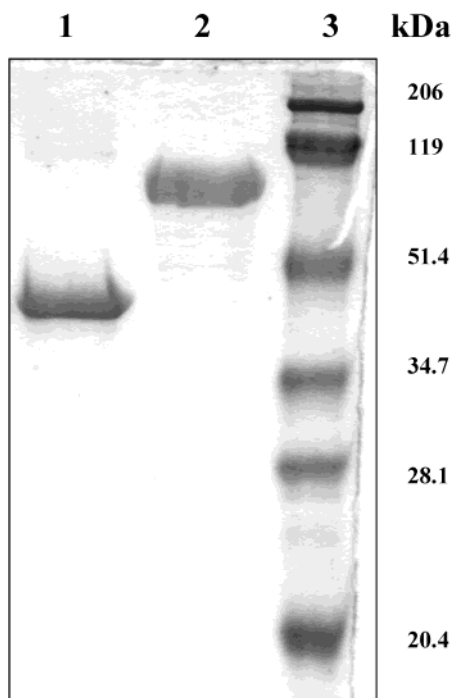
FIGURE 1. SDS−PAGE analysis of purified biopolymers. Lane 1: ELP67MR, Lane 2: ELP153MR, and Lane 3: broad range prestained molecular weight standards. Five micrograms of protein were loaded in each lane.
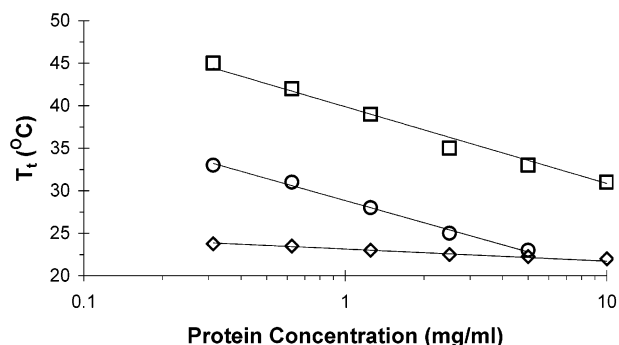

FIGURE 2. Characterization of the phase transition behavior as a function of biopolymer (□) ELP78, (○) ELP153MR, and (◇) ELP67MR concentrations. The value of T_t—temperature of transition—was defined as the temperature at which 50% turbidity occurred. The experiment was carried out in 50 mM degassed acetate buffer at pH 4, using freshly prepared dilutions of protein previously stored as described in materials and methods.

occurred. For all three biopolymers, the phase transition was reversible, and complete resolubilization was observed below the T_t. For the ELP78 biopolymer, the values of T_t were a strong inverse logarithmic function of concentrations (Figure 2). In the presence of the MerR domain, the values of T_t as well as the concentration dependency were significantly reduced. This may be attributed to the increased hydrophobic interactions between the MerR and the ELP67 domains. Contrary to our earlier results with ELP proteins (10), the values of T_t for the longer ELP153MR were consistently higher than the shorter ELP67MR at all concentrations. This difference in transition behaviors may be due to the increased distance between the ELP153 domain and the solvent-exposed hydrophobic regions of MerR. Although the significant reduction in T_t for ELP67MR restricts its utility for field deployment because of aggregation below 25 °C, the unexpected increase in T_t for the longer ELP153MR suggests that this limitation could be easily compensated by increasing
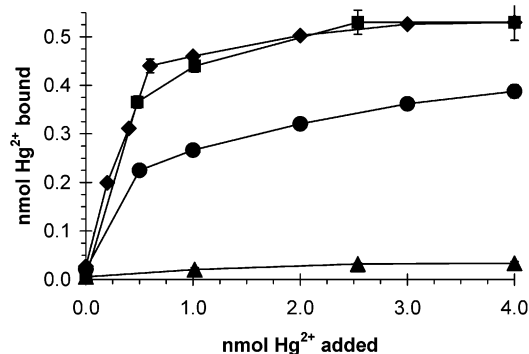

FIGURE 3. Mercury binding stoichiometry of ELP153MR in 50 mM sodium acetate buffer pH 4.0. One nmol of biopolymers (♦) ELP153MR, (■) ELP153MR + 5 mM EDTA, (●) ELP153MR + 5 mM 2-mercaptoethanol, and (▲) ELP78 were mixed with various amounts of mercury. Results represented the average of three sets of experiment with error bars showing the standard deviations.

the chain length of the ELP domain to suit the working temperature range of our interest.

In addition to temperature, the pH behaviors of the biopolymers were investigated. Most industrial wastewaters generated from plating operations are acidic in nature with pH in the range of 3−5. It is important for the biopolymers to retain their phase transition properties within a wide pH range. Both ELP67MR and ELP153MR remained soluble from pH 4−7 (data no shown), and aggregation could be induced by increasing the temperature, demonstrating adaptability of the biopolymers even to acidic environments.

To investigate the recovery efficiency of the biopolymer aggregates by the inverse temperature cycling, supernatants obtained after heat precipitation and centrifugation were analyzed for trace amounts of residual biopolymer by silver staining. The maximum amount of ELP153MR detected was 0.8 ng. This corresponds to greater than 99.96% recovery of the initial 4 mg of added biopolymers by the inverse temperature cycling.

Based on these characterizations and practical considerations, we selected ELP153MR for further mercury binding and recycling studies because of its desired temperature and pH transition behaviors.

Mercury Binding. A major benefit of using metalloregulatory proteins is their high affinity under a wide pH range. We tested the mercury binding by ELP153MR at pH 8.0, 7.0, 6.0, and 4.0. A similar binding stoichiometry of 0.5 mercury per biopolymer was observed at all tested pH values (data not shown), a result consistent with the binding capacity of the native MerR protein, which is one mercury per MerR dimer (20). This result demonstrates that the mercury-binding function and the dimer formation of MerR are not affected by fusion to the ELP domain. Since similar binding behaviors were observed at all pH values, all further experiments were focused on acidic contaminated water at pH 4.0.

To test the kinetics of mercury binding, stoichiometric amounts of ELP153MR and mercury were mixed and incubated from 0 to 60 min. After recovery by precipitation, the amount of Hg^{2+} bound to the aggregates was measured. No difference in the amount of mercury accumulated by ELP153MR was detected between the 0 and 60 min samples, showing that mercury binding to MerR occurred in less than a few seconds required for sample mixing.

The effectiveness of the biopolymers to remove mercury was demonstrated by investigating mercury binding at different Hg^{2+}/biopolymer ratios. Virtually all added mercury was removed from the solution up to a molar ratio of 0.3 Hg^{2+}/biopolymer (Figure 3), suggesting that the MerR-based biopolymer is ideal for treating very low levels of Hg^{2+}
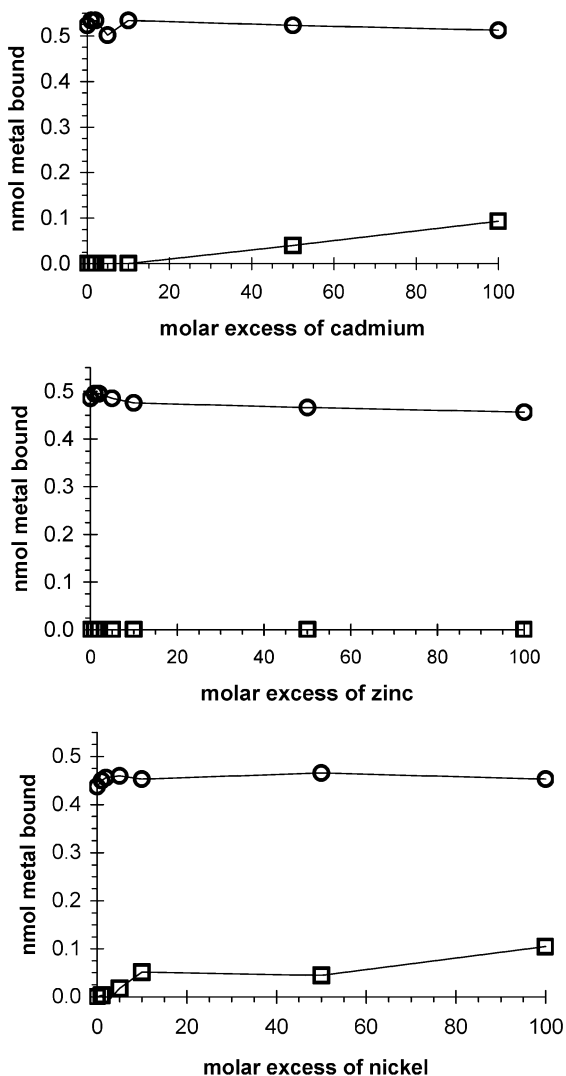
FIGURE 4. Selectivity of the ELP153MR biopolymer. Binding of mercury by ELP153MR in the presence of competing heavy metals. One nmol of biopolymer was mixed with 0.5 nmol of $HgCl_2$ and various amounts of competing heavy metals. The competing metals and mercury were added to the polymer at the same time. The amount of (○) mercury and (□) competing metal bound to the biopolymer is reported.

contamination where other methods are not adequate. In comparison, a similar ELP78 biopolymer was unable to remove an appreciable level of Hg^{2+} from the solution, demonstrating that the ELP moiety itself does not bind or entrap Hg^{2+}.

Selectivity of the Biopolymers. Previously, the MerR protein has been shown to be very specific for mercury binding, but most studies were evaluated on the basis of initiation of DNA transcription by the MerR—metal complex (13). Contradicting reports exist on the selectivity of the MerR protein for direct binding of certain heavy-metals in in vitro systems (21, 22). To demonstrate the in situ binding selectivity of the ELP biopolymers, we investigated whether ELP153MR could bind mercury with the same stoichiometry in the presence of other heavy metals. ELP153MR was mixed with stoichiometric amounts of mercury and up to 100-fold molar excess of cadmium, zinc, or nickel. In all three cases, mercury binding was very specific; only mercury was bound in notable amounts, and the extent of mercury binding was unaffected by the competing heavy metals (Figure 4). The low levels of binding for cadmium and nickel at 100-fold excess is likely due to binding to other cysteine residues not involved in

mercury binding. Since our binding experiments were carried out at pH 4 with no competing thiol present, the binding results reported by other authors (21–23) cannot be directly compared. However, since most contaminated waters are acidic in nature, we believe the reported observations are significant and could be extremely useful for actual remediation.

Many metal chelators and complexing agents are frequently found in contaminated environments at low concentrations. The presence of these agents may compete with the biopolymers for the available mercury. To investigate whether chelators or complexing agents have any effect on Hg^{2+} bioaccumulation, binding isotherms were determined in the presence of 5 mM 2-mercaptoethanol or EDTA. For EDTA, no effect on binding was observed (Figure 3). For mercaptoethanol, ELP153MR bound Hg^{2+} at 80% efficiency at a mercury-to-biopolymer ratio of less than 0.5. Even at higher mercury levels, the biopolymers still retained 60% of the original binding efficiency. The resistance of mercury binding in the presence of EDTA and 2-mercaptoethanol again highlights the high affinity of the MerR domain, which based on DNA transcription initiation studies was reported to be in the nanomolar range in the presence of competing thiols (13). However, the actual binding affinity appears to be significantly higher as the biopolymers bound mercury stronger than EDTA ($K_D = 10^{-25}$ M) (24) and 2-mercaptoethanol ($K_D = 10^{-45}$ M) (13). This high binding affinity is in accordance with the fact that three different cysteine residues from the two MerR subunits are involved in sequestering mercury, which results in a high-affinity tricoordinate mercury binding site (25).

Recycling of ELP153MR for Mercury Removal. The ultimate use of the tunable biopolymers is in a recyclable system of continual mercury binding and extraction. Rapid regeneration of the mercury binding sites is essential in this case. Previously, we have demonstrated that sequestered cadmium could be removed from polyhistidines either by lowering the pH or by treating with EDTA (10). Because of the high affinity of MerR toward mercury, even 100 mM EDTA was unable to extract mercury from the Hg^{2+}—biopolymer complexes at all pH values tested (4–8.8). Only the use of a strong complexing agent, 2-mercaptoethanol, at 50 mM concentration and pH 4 could effectively remove the bound mercury after two rounds of extraction. The regenerated biopolymer aggregates were resolubilized below 25 °C and remained fully functional even after four repeating cycles. In each cycle, the original 219 nM mercury added was reduced to ≤ 8 nM (Figure 5), a concentration below the required drinking water limit of 10 nM (2 ppb).

Mercury Removal from Artificially Contaminated Lake Elsinore Water. The ability of the biopolymers to remove mercury from real water samples rather than buffered solutions was investigated by repeating similar binding experiments with artificially contaminated lake water samples obtained from Lake Elsinore. Table 2 shows the characteristics of the Lake Elsinore water samples used in the study. Unlike buffered solutions, the lake water samples have a pH of 9.3 and high turbidity. Even under this alkaline condition, 99% of the added mercury (219 nM) was removed by the ELP153MR biopolymers (Figure 5). In addition, a similar mercury binding stoichiometry of 0.5 was observed, indicating the presence of suspended solids and other ions in the sample has virtually no effect on mercury binding (data not shown).

Discussion

As regulations on mercury are becoming more stringent, conventional primary treatment methods such as chemical precipitation, carbon adsorption, and ion exchange are inadequate to remove and recover mercury to the required
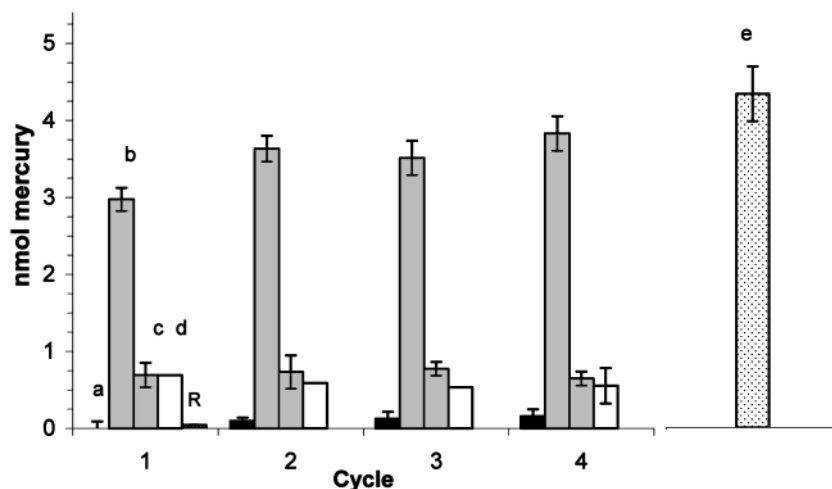
FIGURE 5. Recycling of ELP153MR. In each cycle, 50 nmol of the protein was mixed with 4.36 nmol of mercury. After the first heat precipitation, the amount of residual mercury (a) in the supernatant is shown in the first column. The amount of mercury subsequently extracted from the biopolymers by the first extraction (b) and second (c) extraction step are shown in the second and third columns, respectively. The amount of mercury remained on the biopolymers after double extraction (d) is represented by the last column. The same binding and extraction procedure were repeated four times. The original amount of mercury added in each cycle (e) is shown on the right. A similar removal experiment was performed with artificially contaminated Lake Elsinore water and the amount of mercury remained in the supernatant after binding (R) was measured.

TABLE 2. Characteristics of Lake Elsinore Water

pH	9.3
conductivity (mS/cm)	3.71
alkalinity (meg/L)	11.5
turbidity (NTU)	35
Na^+ (ppm)	370
K^+ (ppm)	21.5
Mg^{2+} (ppm)	62
Ca^{2+} (ppm)	6
Cl^- (ppm)	770
Br^- (ppm)	13.5
SO_4^{2-} (ppm)	217

low concentrations (2 ppb for drinking water) because many mercury-bearing wastes contain substances such as organics, complexing agents, competing heavy metals, and alkaline earth metals that may decrease the metal removal capacity. It becomes clear that secondary processes to selectively remove and recover mercury from dilute waste stream to sub-ppb levels are desirable. Although many chemical ligands offer high affinity toward mercury, their ability to form specific and stable complexes with mercury under adverse pH conditions remains an unsolved problem. In addition, many of the chemical ligands or their decomposed products are hazardous to the environment (26). Biological ligands such as metellothioneins have been investigated as an emerging solution for treating dilute mercury waste (27). Although these cysteine-rich peptides have high affinity for a wide range of heavy metals, they lack the required selectivity and pH range to enable the specific removal and recycling of mercury. An ideal secondary process for mercury cleanup, therefore, requires removal of mercury at low concentrations, flexibility in dealing with adverse environmental conditions, specificity, a low cost operation, and green technology.

We have previously shown that tunable biopolymers composed of ELP fusions to a polyhistidine tag provide an easy and efficient way to recover and recycle sequestered metals by simple environmental triggers (10). However, from a practical point of view, polyhistidines suffer from virtually no selectivity, relatively low affinity to heavy metals, and a narrow working pH range. To provide high specificity and affinity, one could exploit what nature can offer. Many bacteria acquire resistance to heavy metals by triggering the production of transport proteins and enzymes that could actively metabolize and inactivate the toxic effects of these metals (14). The highly specific nature of these resistance mechanisms is the result of a cleverly designed genetic circuit that is tightly controlled by specific metalloregulatory proteins that possess high affinity for the target species.

MerR is a metalloregulatory protein responsible for regulating expression of the mercury detoxification pathway encoded by the mer operon of Tn501. The highly specific nature of MerR against other related heavy metals suggested that it could be used as a superior sorbent enabling the specific removal and recycling of mercury. However, no one today has taken advantage of MerR toward mercury remediation because of the high cost associated with protein purification and mercury recovery. This report demonstrates the coupled utility of the ELP-technology with the high specificity and affinity of MerR dramatically facilitates protein purification and enables specific sequestration and recycling of mercury even in the presence of 100-fold excess of other related heavy metals such as cadmium and zinc. More importantly, the affinity of MerR enables sequestration and removal of mercury to the required EPA levels within a wide range of pHs with affinity exceeding conventional chelators and complexing agents such as EDTA and mercaptoethanol. We believe that the tunable biopolymers reported here offer a practical solution to mercury remediation providing significantly improved affinity, specificity, and cost of operation than conventional secondary treatment methods. An added benefit to this technology is its "green" aspect as no organic solvents and toxic byproducts will be used or generated. As nature offers a wide selection of metalloregulatory proteins (14), a similar strategy could be used to generate ELP-based biopolymers specific for other pollutants such as the currently infamous arsenic and chromium.

Acknowledgments

Literature Cited

(1) Mason, R. P.; Reinfelder, J. R.; Moore, F. M. M. Environ. Sci. Technol. **1996**, 30, 1835−1845.

(2) Young, K.; Ming-Huan, W.; Yung-Shuen, S. *Waste Manage.* **2002**, *22*, 721−726.

(3) Ritter, J. A.; Bibler, J. P. *Water Sci. Technol.* **1992**, *25*, 165−172.

(4) Wagner-Dobler, I.; von Canstein, H.; Li, Y.; Timmis, K. N.; Deckwer, W.-D. *Environ. Sci. Technol.* **2000**, *34*, 4628−4634.

(5) Thompson, J. A.; Jarvinen, G. *Filtr. Separat.* **1999**, *36*, 28−32.

(6) Urry, D. W. *J. Phys. Chem. B.* **1997**, *101*, 11007−11028.

(7) Reiersen, H.; Clarke, A. R.; Rees, A. R. *J. Mol. Biol.* **1998**, *283*, 255−264.

(8) Urry, D. W. et al. *Biopolymers* **1992**, *32*, 1243−1250.

(9) Meyer, D. E.; Chilkoti, A. *Nat. Biotechnol.* **1999**, *17*, 1112−1115.

(10) Kostal, J.; Mulchandani, A.; Chen, W. *Macromolecules* **2001**, *34*, 2257−2261.

(11) Guda, C. et al. *Biotechnol. Lett.* **1995**, *17*, 745−750.

(12) O'Halloran, T.; Walsh, C. *Science* **1987**, *235*, 211−214.

(13) Ralston, D. M.; O'Halloran, T. V. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 3846−3850.

(14) Nies D. H. *Appl. Microbiol. Biotechnol.* **1999**, *51*, 730−750.

(15) Sambrook, J.; Russell, R. W. *Molecular Cloning: A Laboratory Manual*, 3rd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 2001.

(16) Kidd, S. personal communication.

(17) Bennett, P. M.; Grinsted, J.; Choi, C. L.; Richmond, M. H. *Mol. Gen. Genet.* **1978**, *159*, 101−106.

(18) McPherson, D. T.; Xu, J.; Urry, D. W. *Protein Expression Purif.* **1996**, *7*, 51−57.

(19) Laemmli, U. K. *Nature* **1970**, *227*, 680−685.

(20) Shewchuk, L. M.; Verdine, G. L.; Nash, H.; Walsh, C. T. *Biochemistry* **1989**, *28*, 6140−6145.

(21) Corbisier, P. et al. *Anal. Chim. Acta* **1999**, *387*, 235−244.

(22) Klein, J.; Mattes, R. *Anal. Biochem.* **1998**, *260*, 173−182.

(23) Bae, B.; Wu, C.; Kostal, J.; Mulchandai, A.; Chen, W. *Appl. Environ. Microbiol.* **2003**, *69*, 3176−3180.

(24) Martell, A. E.; Smith, R. M. *Critical stability constants*; Plenum Press: New York, 1974.

(25) Helmann, J. D.; Vallard, B. T.; Walsh, C. T. *Science* **1990**, *247*, 946−948.

(26) Matlock, M. M.; Howerton, B. S.; Van Aelstyn, M. A.; Nordstrom, F. L.; Atwood, D. A. *Environ. Sci. Technol.* **2002**, *26*, 1636−1639.

(27) Bae, W.; Mehra, R.; Mulchandai, A.; Chen, W. *Appl. Environ. Microbiol.* **2001**, *67*, 5335−5338.